ORIGINAL

R. A. De Blasi
E. Tonelli
R. Arcioni
M. Mercieri
L. Cigognetti
R. Romano
G. Pinto

In vivo effects on human skeletal muscle oxygen delivery and metabolism of cardiopulmonary bypass and perioperative hemodilution

R. A. De Blasi (✉) · E. Tonelli ·
R. Arcioni · M. Mercieri ·
L. Cigognetti · R. Romano · G. Pinto
Department of Medical-Surgical,
Techno-Biomedical Sciences and
Translational Medicine, Faculty
of Medicine and Psychology,
University "La Sapienza", Rome, Italy
e-mail: radbl@libero.it

Abstract *Purpose:* To investigate the in vivo effects of cardiopulmonary bypass (CPB) and perioperative hemodilution on human skeletal muscle oxygen delivery and metabolism and to determine the dilution state at which these effects arise. *Methods:* We conducted this observational study in adult patients undergoing CPB surgery. Microcirculatory data were obtained by near-infrared spectroscopy from the brachioradial muscle in 20 consecutive patients undergoing hemodilution for CPB. Outcome variables included tissue oxy- and deoxyhemoglobin concentration ([HbO$_2$], [HHb]), oxygen content, blood flow, oxygen delivery, and oxygen consumption. *Results:* Although CPB left tissue blood flow and oxygen delivery unchanged, both microcirculatory variables correlated significantly and inversely with hematocrit (Hct) ($r = -0.39$, $p < 0.001$; $r = -0.50$, $p < 0.001$). CPB also left muscle oxygen consumption (mVO$_2$) unchanged and this variable correlated with the tissue hemoglobin concentration and tissue oxygen delivery ($r = 0.40$, $p = 0.001$; $r = 0.35$, $p = 0.005$). During CPB most of the systemic cardiovascular variables remained unchanged. Conversely at Hct lower than 30%, mean arterial pressure and pH decreased and lactate values increased twofold, whereas microvascular blood volume and oxygen delivery increased. At Hct lower than 20% blood flow and oxygen delivery increased, whereas hemoglobin and oxygen content variables decreased. *Conclusions:* CPB leaves skeletal muscle oxygen delivery and metabolism as measured by near-infrared spectroscopy unchanged. The only factor that correlates directly with the oxygen content variables and inversely with blood flow, and induces significant changes in tissue hemoglobin content and oxygen delivery, is hemodilution.

Keywords Near-infrared spectroscopy · Microcirculation · Cardiopulmonary bypass · Oxygen delivery · Skeletal muscle

Introduction

Although the various complications after cardiopulmonary bypass (CPB) surgery have decreased over time the incidence of organ dysfunction remains unacceptably high. Equally important, organ dysfunction increases the length of intensive care unit (ICU) stay and mortality [1].

The mechanisms leading to organ dysfunction include global hemodynamic and regional blood flow changes, mitochondrial and microcirculatory dysfunction [2–5], and tissue edema [6]. Although the known risk factors for organ dysfunction related to CBP include hemodilution and hypothermia, whether these conditions reduce oxygen tissue delivery and alter cell metabolism remains

controversial. Even though cardiac surgical textbooks indicate hemodilution as an important factor in minimizing microcirculatory disturbances secondary to increased blood viscosity and adverse rheologic effects due to low temperatures [7, 8], no in vivo evidence yet shows whether hemodilution by lowering macrovascular blood hemoglobin content alters microcirculatory oxygen delivery and cellular metabolism.

Few studies have directly examined the microcirculation during CPB in humans [9, 10]. None have supplied in vivo data on skeletal muscle changes in microcirculatory oxygen delivery and tissue metabolism and, most importantly, nor do we have a specific variable for measuring oxygen delivery online in patients with suspected microcirculatory dysfunction. A validated, noninvasive, reliable, bedside technique for directly investigating skeletal muscle microcirculatory oxygen delivery and cellular metabolism in vivo is quantitative near-infrared spectroscopy (NIRS) during venous occlusion [10–13]. An appropriate method for quantifying tissue hemoglobin concentrations is phase-modulation spectroscopy because it measures CPB and hemodilution-induced changes in tissue photon path-lengths [14]. Having online information on changes in hemoglobin concentrations, blood flow, oxygen content, delivery, and consumption, and possible interactions between these microcirculatory variables and cardiocirculatory and metabolic variables during hemodilution might help in undertaking therapeutic strategies to adjust them.

We designed this noninvasive observational study first to investigate directly in patients' skeletal muscle the in vivo effects of CPB and perioperative hemodilution on tissue oxygen delivery and tissue metabolism. Second, we investigated the role of temperature and major systemic cardiocirculatory and metabolic variables on microcirculatory variables as measured by phase-modulation NIRS in patients undergoing CPB surgery. We also noninvasively investigated how hematocrit (Hct) in the same patients influences oxygen delivery and tissue metabolism. As major NIRS variables to do so we assessed the oxy- and deoxyhemoglobin concentration ([HbO$_2$], [HHb]), muscle blood flow (mBF), oxygen content in tissue (CtO$_2$), tissue oxygen saturation (StO$_2$), and muscle oxygen consumption (mVO$_2$). To test a new online index for measuring oxygen availability at the bedside we used NIRS to measure the sudden increase in [HbO$_2$] after venous occlusion during and after CPB.

Methods

During a study conducted at the Sant' Andrea Hospital, an academic center, from January 2010 to July 2011 we enrolled 20 adult patients scheduled for elective cardiac surgery (coronary artery bypass) with the use of CPB. The institutional review board approved the study procedures and each patient gave written informed consent before inclusion. Exclusion criteria are reported in Online Resource 1.

Anesthesia and perioperative management

After oral benzodiazepine premedication, on the patient's arrival in the operating room, arterial and vein lines were placed before inducing anesthesia. Anesthetic management and perioperative procedures are given in Online Resource 2.

CPB management is reported in Online Resource 3. The body temperature was allowed to drift to a minimum temperature from 33 to 35°C. When CPB ended patients were actively rewarmed slowly to approximately 36°C with warm forced-air convection. Clinical severity was assessed preoperatively with the logistic EuroSCORE and the risk of mortality was classified as low (score 1–2), medium (score 3–5), and high (score >6) [15].

Time points for data collection

Data for systemic cardiovascular and metabolic and microcirculatory variables were collected 20–30 min after anesthesia induction (baseline), soon after CPB started (CPB1), 40 min after CPB started (CPB2), and 30 min after recovery from CPB (no CPB).

Microcirculatory data collected

Microcirculatory variables were measured with a phase-modulation NIR spectrophotometer (ISS Oximeter model 96208, Illinois, USA), as described in detail elsewhere [16], performing procedures described in Online Resource 4. Two sets of NIRS measurements were recorded at each time point. A first set of static data was collected by directly measuring the stable tracks of the oxygenated and the deoxygenated forms of tissue hemoglobin concentration immediately before pneumatic compression. A second set of dynamic variables were obtained by measuring tissue hemoglobin concentration changes after repeated pneumatic cuff inflations [17, 18] (Table 1). How NIRS variables were obtained is reported in Online Resource 5.

To assess how Hct influences cardiovascular systemic and microcirculatory variables we compared data corresponding to Hct values higher and lower than 30% and higher and lower than 20%.

Table 1 List of NIRS variables and types of measurement and calculation

Abbreviations	Type of measurement	Type of calculation	Units
$[HbO_2]$	Direct	–	μmol/l
$[HHb]$	Direct	–	μmol/l
$[HbT]$	Calculated	$[HbO_2] + [HHb]$	μmol/l
CtO_2	Calculated	$1.34 \times StO_2/100 \times ([HbT] \times 0.645/100)$	ml O_2/100 ml
StO_2	Calculated	$([HbO_2]/([HbO_2] + [HHb])) \times 100$	%
tBV	Calculated	$[HbT]$ in relation to the endovascular Hb	ml/100 ml
tDO_2	Calculated	$CtO_2 \times mBF$	ml O_2/min/100 ml
$tHbO_2D$	Calculated	Rapid $[HbO_2]$ increase during venous occlusion	ml O_2/min/100 ml
mBF	Calculated	Rapid $[HbT]$ increase during venous occlusion corrected for endovascular Hb	ml/min/100 ml
mVO_2	Calculated	Stable and prolonged $[HHb]$ increase during venous occlusion	ml O_2/min/100 ml

$[HbO_2]$ oxygenated hemoglobin concentration, $[HHb]$ deoxygenated hemoglobin concentration, $[HbT]$ total hemoglobin concentration, CtO_2 tissue oxygen content, StO_2 tissue oxygen saturation, tBV tissue blood volume, tDO_2 tissue oxygen delivery, $tHbO_2D$ tissue oxyhemoglobin delivery, mBF muscular blood flow, mVO_2 muscular oxygen consumption

Statistical analysis

The Kolmogorov–Smirnov test was used to assess normal distribution followed by repeated measurements analysis of variance (ANOVA). Bonferroni's test was used to establish differences between the four data collection points. All data are expressed as mean ± standard deviation (SD). A one-way ANOVA was used to test differences in the variables grouped according to the patients' severity score and degree of hemodilution. The nonparametric Mann–Whitney test was used to analyze non-normally distributed data. Pearson's correlation test and regression analysis were used to determine a possible correlation between two variables. p values less than 0.05 were considered as statistically significant. Data were analyzed with the software program MedCalc, version 11.5.

Table 2 Demographic, anthropometric data, clinical severity, and comorbidities for the 20 patients undergoing cardiac surgery

Demographic and anthropometric data	
Sex, n, M/F	13/7
Age, mean (SD), years	68.2 (7.5)
BMI, median (IQR), (kg/m^2)	26.0 (25.0–27.4)
Logistic EuroSCORE, median (IQR)	8.2 (3.8–13.0)
Low risk, n	3
Medium risk, n	5
High risk, n	12
Comorbidities, n (%)	
Hypertension	12 (60.0)
Hyperlipidemia	10 (50.0)
Chronic obstructive pulmonary disease	4 (20.0)
Diabetes mellitus	6 (30.0)
Hypothyroidism	3 (15.0)
Chronic renal failure	2 (10.0)
Peripheral arterial disease	3 (15.0)

BMI body mass index, *IQR* interquartile range

Results

All 20 patients enrolled completed the study (Table 2). All cardiocirculatory and microcirculatory variables tested at each time point were normally distributed.

Temperature and systemic cardiocirculatory and metabolic variables

Temperature diminished by about a mean 6% after CPB (range 1.4–14.0%) and increased after patients were warmed.

Throughout surgery patients were kept hemodynamically stable with inotropic and vasoconstrictor agents and no major hypotensive episodes developed during the study. The only inotropic drug used during CPB was enoximone (Table 3). None of the patients required vasoconstrictors.

All the tested systemic cardiovascular variables remained unchanged during CPB except mean arterial pressure (MAP). MAP varied markedly between subjects and at CPB2 diminished significantly from baseline (Table 3). MAP variations correlated with those in Hct ($r = 0.37$, $p = 0.002$). The cardiac index (CI) underwent slight individual variations during pre- and post-CPB reaching values similar to those related to pump flow. SvO$_2$ invariably remained at values exceeding 70%. When CPB began, Hct diminished by about 45% (range 23.2–63.4%) and remained lower than baseline also when CPB ended. Hb also underwent a CPB-induced decrease but to a lesser extent (23%). Repeated measurements ANOVA showed that the mean corpuscular hemoglobin concentration (MCHC) (Hb/Hct ratio) increased significantly after CPB ($p < 0.001$) and decreased after CPB ended but remained at values higher than baseline.

The metabolic variable pH remained in the normal range during CPB and no correlation was found between changes in pH and changes in other variables measured.

Table 3 Temperature and systemic cardiocirculatory and metabolic variables measured in the 20 patients after anesthesia induction, twice during CPB and 30 min thereafter

Variable	Baseline	CPB1	CPB2	No CPB	p value
Mean arterial pressure (mmHg)	79.83 (13.2)	67.7 (11.1)	64.12[a] (15.0)	66.5 (11.0)	0.003
Cardiac index (l/min/m^2)	2.0 (1.1)	2.3 (0.9)	2.3 (1.3)	2.5 (0.8)	0.356
Central venous pressure (mmHg)	8.9 (4.7)	8.1 (4.1)	8.7 (3.6)	9.5 (3.2)	0.533
Temperature (°C)	35.8 (0.6)	33.9[b,c] (1.6)	34.7[d,e] (1.3)	36.0 (0.5)	<0.001
Hematocrit (%)	35.2 (4.6)	21.1[f] (3.6)	21.6[g,h] (3.8)	25.6[i] (6.7)	<0.001
Hemoglobin concentration (g/dl)	11.6 (1.3)	8.8[j] (1.3)	8.7[k] (1.5)	9.7[l] (2.0)	<0.001
Lactates (mmol/l)	0.64 (0.29)	1.29[m] (0.55)	1.48[n] (0.59)	1.58[o] (0.70)	<0.001
pH	7.44 (0.04)	7.43 (0.06)	7.40 (0.07)	7.41 (0.05)	0.077
pCO$_2$	34.9 (4.9)	37.5 (5.8)	40.4[p] (4.0)	38.3 (4.3)	0.004
Mixed venous oxygen saturation (%)	77.3 (7.6)	85.1 (11.3)	79.3 (9.2)	74.3 (9.7)	0.841
Inotropic drug requirements, n, dose range (µg/kg/min)	EN, 14 3–5 µg/kg/min	EN, 18 3–5 µg/kg/min	EN, 18 3–5 µg/kg/min	EN, 9 3–5 µg/kg/min	–

Data were expressed as mean ± SD

CPB1 soon after the start of CPB, *CPB2* 40 min after the start of CPB, *EN* enoximone, *n* number of subjects, *ANOVA* analysis of variance

ANOVA for repeated measurements was used to compare data. Bonferroni's test was used for post hoc comparison to identify significant variations: [a] $p = 0.049$ versus baseline; [b] $p < 0.001$ versus baseline; [c] $p < 0.001$, [d] $p < 0.001$ versus with no CPB; [e] $p = 0.0198$; [f] $p < 0.001$, [g] $p < 0.001$, [i] $p < 0.001$ versus baseline; [f] $p = 0.0123$, [h] $p = 0.0198$ versus with no CPB; [j] $p < 0.001$, [k] $p < 0.001$, [l] $p = 0.0013$ versus baseline; [m] $p < 0.001$, [n] $p < 0.001$, [o] $p < 0.001$ versus baseline; [p] $p = 0.002$ versus baseline

Conversely, PaCO$_2$ increased at the second time point during CPB from baseline and lactates increased after CPB began until patients were weaned from CPB but values remained below 2.5 mmol/l (Table 3). Lactate values showed a weak inverse correlation with changes in Hct ($r = -0.28$, $p = 0.015$) and MAP ($r = -29$, $p = 0.025$).

Microcirculatory data in skeletal muscle tissue

About 10% of NIRS tracks recorded were unreliable and discarded. The variability in NIRS triplicate measurements remained within an acceptable range (mean coefficient of variation 11.4, ranging from 2 to 28%).

Hemoglobin concentration and blood volume

[HbO$_2$] values diminished by 14% from baseline after CPB (Bonferroni test, $p < 0.001$) and remained lower than baseline even after CPB ended. Conversely, [HHb] invariably remained unchanged ($p = 0.824$). Consequently the [HbT] decreased by 8.5% from baseline during CPB and remained low when CPB ended (Table 4).

Pearson's test identified a weak correlation between [HbT] and Hct ($r = 0.23$, $p = 0.044$) or MAP ($r = 0.29$, $p = 0.016$) but no correlation between [HbT] and CI, temperature, pH, PaCO$_2$, lactate, or the microcirculatory variable mBF. Microcirculatory tBV increased significantly from baseline during CPB (Table 4). tBV increment correlated with mBF ($r = 0.40$, $p = 0.001$),

Table 4 Microcirculatory variables measured by NIRS in the 20 patients after anesthesia induction, twice during CPB and 30 min thereafter

Variables	Baseline	CPB1	CPB2	No CPB	p value
[HbO$_2$] (µmol/l)	35.3 (9.8)	30.3[a] (10.0)	31.9[b] (9.5)	29.1[c] (8.5)	<0.001
[HHb] (µmol/l)	19.9 (4.7)	20.3 (5.5)	19.8 (4.7)	19.5 (5.7)	0.824
[HbT] (µmol/l)	55.2 (12.8)	50.5[d] (11.6)	51.7[e] (12.1)	48.6[f] (11.1)	<0.001
CtO$_2$ (ml O$_2$/100 ml)	0.31 (0.08)	0.26[g] (0.09)	0.28[h] (0.08)	0.25[i] (0.07)	<0.001
StO$_2$ (%)	63.2 (8.1)	61.9 (11.6)	61.2 (7.1)	59.7 (8.7)	0.064
tBV (ml/100 ml)	3.1 (0.8)	3.8[j] (0.9)	3.9[k] (0.8)	3.4 (0.9)	<0.001
tHbO$_2$D (ml O$_2$/min/100 ml)	1.4 (0.8)	2.1 (0.9)	2.2 (1.2)	1.9 (0.9)	0.060
tDO$_2$ (ml O$_2$/min/100 ml)	0.12 (0.07)	0.15 (0.09)	0.14 (0.13)	0.11 (0.10)	0.614
mBF (ml/min/100 ml)	0.39 (0.17)	0.55 (0.32)	0.51 (0.41)	0.43 (0.28)	0.274
mVO$_2$ (ml O$_2$/min/100 ml)	0.14 (0.09)	0.16 (0.07)	0.20 (0.12)	0.20 (0.15)	0.312

Data are expressed as mean (SD). All abbreviations are defined in Tables 1 and 3

ANOVA was used to compare data for repeated measurements. Post hoc statistical comparison with Bonferroni's test was used to identify significant variations: [a] $p = 0.011$, [b] $p = 0.014$, [c] $p < 0.001$ versus baseline; [d] $p = 0.001$, [e] $p = 0.005$, [f] $p = 0.002$ versus baseline; [g] $p = 0.002$, [h] $p = 0.012$, [i] $p < 0.001$ versus baseline; [j] $p < 0.001$, [k] $p < 0.001$ versus baseline
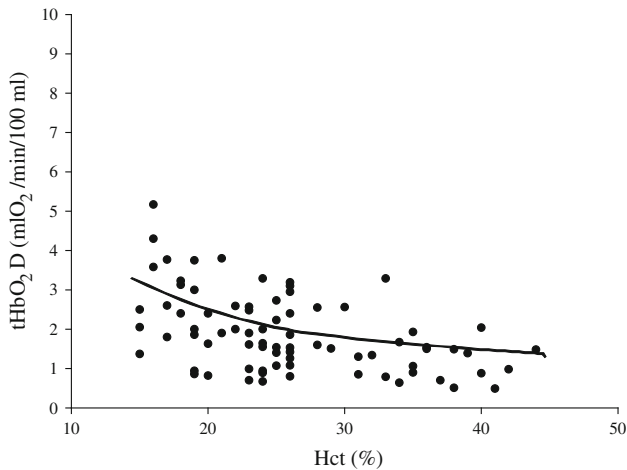
Fig. 1 Scattergram showing tissue oxyhemoglobin delivery (tHbO$_2$) values and percentage changes in systemic hematocrit (Hct%) in the 20 patients undergoing perioperative hemodilution during CPB surgery. Note the regression line showing a nonlinear relation between the two variables described by the equation $y = 5.45 - 0.21x + 0.0026x^2$

tHbO$_2$D ($r = 0.52$, $p < 0.001$), and mVO$_2$ ($r = 0.46$, $p < 0.001$).

Tissue oxygenation, blood flow, and oxygen delivery

CtO$_2$ decreased by about 14% after CPB began and remained low after CPB ended. Despite these changes, StO$_2$ remained unchanged throughout the study. CtO$_2$ correlated with Hct and mVO$_2$ ($r = 0.25$, $p = 0.028$; $r = 0.44$, $p < 0.001$) but not with changes in mBF, oxygen delivery, or changes in systemic cardiocirculatory variables such as CI, pH, lactate, and MAP. Pearson's test showed no correlations between StO$_2$ and Hct, or between StO$_2$ and skeletal muscle blood flow or mVO$_2$.

Although the microcirculatory blood flow and oxygen delivery expressed as tDO$_2$ and tHbDO$_2$ remained statistically unchanged during the study (Table 4), mBF, tDO$_2$, and tHbDO$_2$ were inversely correlated with Hct ($r = -0.39$, $p < 0.001$; $r = -0.27$, $p = 0.042$ and $r = -0.50$, $p < 0.001$). Hct and tHbDO$_2$ had a nonlinear relationship (Fig. 1).

tHbO$_2$D, the new online variable expressing tissue oxygen delivery, correlated with tDO$_2$ ($r = 0.77$, $p < 0.001$).

Muscle oxygen consumption

mVO$_2$ remained almost unchanged during CPB. mVO$_2$ correlated directly with [HbO$_2$] ($r = 0.44$, $p < 0.001$) and with [HHb] ($r = 0.39$, $p = 0.001$). mVO$_2$ also correlated with the microcirculatory variables tDO$_2$ ($r = 0.47$, $p < 0.001$), tHbO$_2$D ($r = 0.35$, $p < 0.005$), mBF ($r = 0.31$, $p = 0.014$), and with systemic lactates ($r = 0.31$, $p = 0.012$). No correlation was found between mVO$_2$ and MAP, CI, pH, PaCO$_2$, Hct, or temperature.

Influence of hematocrit on systemic cardiovascular and local microcirculatory variables

The one-way ANOVA testing differences in the measured variables grouped according to the severity of the patients' clinical conditions disclosed no differences among groups even when we pooled data for low- and medium-risk subjects. Conversely, nearly all the tested variables changed significantly according to Hct. ANOVA showed that at Hct values less than 30% the systemic variables MAP and pH decreased and PaCO$_2$ and lactates increased by 10 and 44%, whereas the only microcirculatory variables that increased were tBV and tHbO$_2$D (Table 5). At an Hct cutoff point below 20%, MAP invariably diminished, whereas the other systemic variables remained unchanged. The microcirculatory hemoglobin and oxygen content variables all diminished in relation to Hct, whereas blood flow and oxygen delivery increased (Table 5). The only microcirculatory variables that remained unchanged despite variations in Hct were StO$_2$ and mVO$_2$.

Data on the patients' postoperative course are given in Online Resource 6.

Discussion

The first finding in this noninvasive observational study is that although as expected CPB and perioperative hemodilution reduce tissue hemoglobin and oxygen content as measured by the microcirculatory variables [HbT] and CtO$_2$, they have no documentable in vivo effect on patients' skeletal muscle tissue oxygen delivery and tissue metabolism. This new finding adds to current knowledge on in vivo CPB-related microcirculatory changes within skeletal muscle. We also confirm findings for other techniques or other tissues showing that neither temperature nor any of the major systemic cardiocirculatory and metabolic variables influence microcirculatory function in patients undergoing CPB surgery [2, 5] and support those in sepsis [19]. Overall the observed microcirculatory changes related to Hct indicate the Hct level below which compensatory mechanisms probably intervene so that the physician can undertake therapeutic strategies to adjust them. Our study also provides in vivo information on how CPB maintains constant oxygen delivery and tissue metabolism.

Table 5 Comparison of variables grouped for different hematocrit values in the 20 patients

	Hct > 30% (n = 22)	Hct < 30% (n = 56)	p value	Hct > 20% (n = 60)	Hct < 20% (n = 18)	p value
Systemic cardiocirculatory and metabolic variables						
MAP (mmHg)	77.1 (12.8)	67.4 (13.3)	0.003	72.4 (13.6)	62.0 (10.9)	0.010
CI (l/min/m^2)	2.52 (0.90)	2.54 (0.64)	0.876	2.43 (0.69)	2.50 (0.72)	0.506
pH	7.45 (0.05)	7.42 (0.06)	0.019	7.43 (0.05)	7.44 (0.06)	0.382
PaCO$_2$	35.3 (5.2)	38.7 (4.8)	0.020	38.5 (5.1)	35.5 (4.6)	0.060
Lactates (mmol/l)	0.9 (0.6)	1.3 (0.7)	0.015	1.4 (0.8)	1.5 (0.6)	0.789
SvO$_2$ (%)	76.5 (6.2)	77.1 (12.0)	0.962	76.3 (10.0)	79.0 (9.8)	0.411
Microcirculatory variables measured by NIRS						
[HbO$_2$] (μmol/l)	34.7 (10.2)	30.4 (9.2)	0.077	32.9 (10.1)	27.3 (5.7)	0.019*
[HHb] (μmol/l)	19.9 (4.9)	19.8 (5.2)	0.917	20.4 (5.1)	18.1 (4.6)	0.018
[HbT] (μmol/l)	54.7 (13.2)	50.6 (11.2)	0.143	53.4 (12.3)	45.3 (7.8)	0.015
tBV (ml/100 ml)	3.0 (0.8)	3.7 (0.9)	0.002	3.4 (0.9)	4.0 (0.7)	0.015
CtO$_2$ (ml O$_2$/100 ml)	0.30 (0.09)	0.26 (0.08)	0.078	0.28 (0.09)	0.23 (0.05)	0.010*
StO$_2$ (%)	62.7 (8.3)	59.7 (9.5)	0.201	59.7 (12.4)	59.3 (8.2)	0.891
mBF (ml/min/100 ml)	0.38 (0.25)	0.50 (0.33)	0.132	0.40 (0.23)	0.69 (0.40)	0.002*
tDO$_2$ (ml O$_2$/min/100 ml)	0.12 (0.09)	0.13 (0.10)	0.485	0.12 (0.08)	0.17 (0.13	0.030
tHbO$_2$D (ml O$_2$/min/100 ml)	1.3 (0.7)	2.1 (1.0)	<0.001*	1.7 (0.8)	2.7 (1.2)	0.002*
mVO$_2$ (ml O$_2$/min/100 ml)	0.16 (0.13)	0.17 (0.11)	0.674	0.17 (0.12)	0.18 (0.10)	0.745

Data are expressed as mean and (SD). One-way ANOVA was used to compare data

NIRS near-infrared spectroscopy, *MAP* mean arterial pressure, *CI* cardiac index, *SvO$_2$* mixed venous saturation, *n* number of measurements; other abbreviations are defined in Table 1

* Mann–Whitney test

Hemoglobin concentration and oxygen content in skeletal muscle

Some help in interpreting the reduced [HbT] we measured by NIRS in patients undergoing CPB surgery comes from the fact this variable reflects the endovascular Hb concentration and microvascular changes including the number of capillaries, arteriovenous shunts, postcapillary venules, and to a lesser extent the arterioles. Calculating tBV in part helps to distinguish the [HbT] portion linked to changes in the tissue microvascular bed. During hemodilution, NIRS showed higher tissue [HbT] values than the reduced endovascular Hb concentration led us to expect, possibly because Hct became less homogenously distributed even within the capillary network [20] or because the microvascular bed increased [21]. Besides confirming that tBV increased during CPB our NIRS findings show that Hct correlates only with [HbT] and not with the single HbO$_2$ and HHb variables, implying that the CPB-induced changes in microvascular bed distribution differ in the individual patients. Our results suggest that the increased microvascular bed during CPB also influences tissue oxygen extraction.

Measuring CtO$_2$ should prove clinically useful because it provides a single variable that synthesizes the final effect of changes in Hct on tissue hemoglobin saturation and concentration. In our study, the increasing blood flow and oxygen delivery presumably counterbalance the reduced CtO$_2$ content related to endovascular Hb during CPB, leaving tissue metabolism almost unchanged. Owing to CPB-induced changes in tissue blood volume, changes in CtO$_2$ behave differently from changes in StO$_2$. The finding that the microcirculatory variable StO$_2$ remains unchanged from baseline despite the reduced CtO$_2$ and Hct could again be explained by concurrent CPB-induced changes in the individual vascular bed (tBV) and in mVO$_2$. Possible reasons why these microcirculatory changes outlasted CPB are that Hct remained low or inflammatory factors linked to CPB persisted [2].

Microvascular perfusion and oxygen delivery and consumption

Even if our in vivo NIRS findings show that CPB and perioperative hemodilution leave skeletal muscle blood flow and oxygen delivery unchanged the relationship between changes in the microcirculatory variables shows that mBF and tHbO$_2$D changes depend on microvascular bed volume expressed by tBV and on the endovascular Hb concentration. Our NIRS data on tissue blood flow agree with the correlation between the normovolemic reduction in the erythrocyte count and skeletal muscle blood flow despite unchanged oxygen delivery [22, 23]. Oxygen delivery presumably remains constant during hemodilution either because the microvascular bed increases or the erythrocyte circulation time diminishes. Ample evidence underlines the importance of erythrocytes in blood flow control at rest [24, 25]. Others have also underlined the important role of hemoglobin oxygenation and to a lesser extent the total erythrocyte count

and Hb molecules in regulating skeletal muscle blood flow and oxygen delivery [22]. In our study the mild temperature fall during CPB surgery presumably made it unnecessary to reduce Hct-induced viscosity. Although CPB and perioperative hemodilution leave tissue oxygen consumption unchanged, individual changes in mVO_2 are directly related to changes in CtO_2, microvascular bed volume, blood flow, and oxygen delivery and affect the systemic lactate concentration. These NIRS findings therefore provide further evidence that oxygen extraction changes according to skeletal muscle blood flow and oxygen delivery as others have observed indirectly [22]. Because the time courses for the variables used to measure blood flow and oxygen consumption differ and static measures cannot interfere with dynamic measures, these calculations excluded possible mathematical coupling between oxygen delivery and consumption.

Influence of Hct on microvascular variables

Our data confirm what others observed indirectly in skeletal muscle tissue [22], namely that hemodilution reduces the total hemoglobin tissue concentration and blood flow but only at low Hct values (<20%) even though microvascular bed volume and oxygen delivery increased at higher Hct values (Table 5). Despite remaining within a normal range, plasma lactate values, pH, and $PaCO_2$ are most sensitive to Hct values less than 30% and correlate with the low MAP during CPB. Why the MCHC changed so dramatically during CPB remains an interesting question for future research.

The new online index for measuring oxygen delivery

Our finding that oxygen tissue delivery can be expressed either by calculating the variables [HbT], StO_2, and mBF (tDO_2), or simply and more directly by calculating the sudden linear increase in HbO_2 after venous occlusion ($tHbO_2D$) receives support from the good correlation between the two variables even though $tHbO_2D$ is more sensitive than tDO_2 to changes in plasma Hct and also varies significantly as Hct goes below 30%.

Effect of temperature and systemic variables on microcirculation

Other factors may have influenced the microcirculation, including the increase in inflammatory mediators [26] and the decrease in core temperature. Despite the known microcirculatory effect induced by marked hypothermia [27], the effect induced by moderate hypothermia is controversial. In our patients undergoing CPB surgery spontaneous changes in core temperature had no influence on any of the studied variables. Presumably the slight temperature decrease during CPB had no effect on tissue oxygen consumption either because the effects induced by anesthetic drugs predominated or the effect of temperature-induced changes were counterbalanced by inotropic drugs such as enoximone.

Our study also confirms that global hemodynamic variables have no influence on microcirculation in patients undergoing CPB [6, 28]. Serum lactates seem weakly sensitive to changes in MAP and Hct even if our patients' values remained within normal ranges and none of the patients had organ failure after CPB.

Limitations

Our study has several limitations. First, a possible technical limitation is that the changes in the microvascular bed and blood flow we describe are indirectly derived from changes in tissue Hb related to Hb content in blood samples. Besides, microcirculatory Hct is nonhomogeneously distributed and cannot be predicted owing to complex factors that include variations in erythrocyte volume and vascular smooth muscle activity [29].

Second, even though we detected a statistical relationship only between microcirculatory variables as measured by NIRS and major systemic cardiocirculatory and metabolic variables and especially with Hct, microvascular perfusion reflects other factors, e.g., artificial nonpulsatile perfusion by CPB, blood contact with artificial surfaces, surgical trauma, leukocyte activation, and CPB-related inflammatory factors beyond the purpose of this study.

Finally, because we compared variables measured after anesthesia induction with those measured during and after CPB the NIRS microcirculatory changes we observed are presumably almost unaffected by anesthetic drugs but might partly reflect changes induced by endogenous catecholamine release or variations in the depth of anesthesia.

Conclusions

This noninvasive observational NIRS study gives new insights into tissue oxygen delivery and metabolic changes and compensatory mechanisms during CPB and perioperative hemodilution. During CPB surgery patients' skeletal muscle oxygen delivery and metabolism remain almost unchanged. The only factor that correlates directly with the oxygen content variables and inversely with blood flow and induces significant changes in tissue hemoglobin content, blood volume, and oxygen delivery

leaving oxygen consumption unchanged is hemodilution. Hence hemodilution in patients undergoing CPB should be kept within levels unlikely to induce microcirculatory changes. Another clinically useful finding is that the sudden increase in $[HbO_2]$ after venous occlusion $(tHbO_2D)$ promises to be a practical new online index for measuring by NIRS oxygen availability at the bedside in patients with suspected microcirculatory dysfunction.

References

1. Patila T, Kukkonen S, Vento A, Pettila V, Suojaranta-Ylinen R (2006) Relation of the sequential organ failure assessment score to morbidity and mortality after cardiac surgery. Ann Thorac Surg 82:2072–2078
2. Bauer A, Kofler S, Thiel M, Eifert S, Christ F (2007) Monitoring of the sublingual microcirculation in cardiac surgery using orthogonal polarization spectral imaging. Anesthesiology 107:939–945
3. Doerschug KC, Delsing AS, Schmidt GA, Haynes WG (2007) Impairments in microvascular reactivity are related to organ failure in human sepsis. Am J Physiol Heart Circ Physiol 293:H1065–H1071
4. den Uil CA, Lagrand WK, Spronk PE, Spronk PE, van Domburg RT, Hofland J, Lüthen C, Brugts JJ, van der Ent M, Simoons ML (2008) Impaired sublingual microvascular perfusion during surgery with cardiopulmonary bypass: a pilot study. J Thorac Cardiovasc Surg 136:129–134
5. De Backer D, Dubois MJ, Schmartz D, Schmartz D, Marc Koch, Anne Ducart, Luc Barvais, Vincent JL (2009) Microcirculatory alterations in cardiac surgery: effects of cardiopulmonary bypass and anesthesia. Ann Thorac Surg 88:1396–1403
6. Riddington DW, Venkatesh B, Boivin CM, Bonser RS, Elliott TSJ, Marshall T, Mountford PJ, Bion JF (1996) Intestinal permeability, gastric intramucosal pH, and systemic endotoxemia in patients undergoing cardiopulmonary bypass. JAMA 275:1007–1012
7. Kirklin JW, Barrat-Boyes BG (1993) Hypothermia, circulatory arrest, and cardiopulmonary bypass. In: Cardiac surgery. Churchill Livingstone, New York, pp 62–73
8. Cooper MM, Elliott MJ (1994) Cardiopulmonary bypass in neonates, infants and young children. In: Jonas RA, Elliott MJ (eds) Haemodilution. Butterworth-Heinemann, Oxford, pp 82–89
9. Trzeciak S, McCoy JV, Phillip Dellinger R, Arnold RC, Rizzuto M, Abate NL, Shapiro NI, Parrillo JE, Hollenberg SM (2008) Early increases in microcirculatory perfusion during protocol-directed resuscitation are associated with reduced multi-organ failure at 24 h in patients with sepsis. Intensive Care Med 34:2210–2217
10. De Blasi RA, Palmisani S, Alampi D, Mercieri M, Romano R, Collini S, Pinto G (2005) Microvascular dysfunction and skeletal muscle oxygenation assessed by phase-modulation near infrared spectroscopy in patients with septic shock. Intensive Care Med 31:1661–1668
11. De Blasi RA, Luciani R, Punzo G, Arcioni R, Romano R, Boezi M, Menè P (2009) Microcirculatory changes and skeletal muscle oxygenation measured at rest by non-infrared spectroscopy in patients with and without diabetes undergoing haemodialysis. Crit Care 13(5):S9–S19
12. Homma S, Eda H, Ogasawara S, Kagaya A (1996) Near infrared estimation of O_2 supply and consumption in forearm muscles working at varying intensity. J Appl Physiol 80:1279–1284
13. Van Beekvelt MCP, Colier WNJM, Van Engelen BGM, Hopman MTE, Wevers RA, Oeseburg B (1998) Validation of measurement protocols to assess oxygen consumption and blood flow in the human forearm by near infrared spectroscopy. In: Benaron DA, Chance B, and Ferrari M (eds) Photon propagation in tissues. III. Proc SPIE 3194:133–144
14. Yoshitani K, Kawaguchi M, Okuno T, Kanoda T, Ohnishi Y, Kuro M, Nishizawa M (2007) Measurements of optical pathlength using phase-resolved spectroscopy in patients undergoing cardiopulmonary bypass. Anesth Analg 104:341–346
15. Roques F, Michel P, Goldstone AR, Nashef SA (2003) The logistic EuroSCORE. Eur Heart J 24(9):882–883
16. Fantini S, Franceschini MA, Maier JS, Walker SA, Barbieri B, Gratton E (1995) Frequency domain multichannel optical detector for non-invasive tissue spectroscopy and oxymetry. Opt Eng 34:32–42
17. De Blasi RA, Ferrari M, Natali A, Conti G, Mega A, Gasparetto A (1994) Noninvasive measurement of forearm blood flow and oxygen consumption by near infrared spectroscopy. J Appl Physiol 76:1388–1393
18. Van Beekvelt M, Borghuis M, Van Engelen B, Wevers R, Collier W (2001) Adipose tissue thickness affects in vivo quantitative near-IR spectroscopy in human skeletal muscle. Clin Sci (Lond) 101:21–28
19. De Backer D, Creteur J, Dubois MJ, Sakr Y, Koch M, Verdant C, Vincent JL (2006) The effects of dobutamine on microcirculatory alterations in patients with septic shock are independent of its systemic effects. Crit Care Med 34:403–408
20. Gaehtgens P (1984) 'Regulation' of capillary haematocrit. Int J Microcirc Clin Exp 3(2):147–160
21. Lindbom L, Mirhashemi S, Intaglietta M, Arfors KE (1988) Increase in capillary blood flow and relative haematocrit in rabbit skeletal muscle following acute normovolaemic anaemia. Acta Physiol Scand 134(4):503–512
22. Gonzàlez-Alonso J, Mortensen SP, Dawson EA, Secher NH, Damsgaard R (2006) Erythrocytes and the regulation of human skeletal muscle blood flow and oxygen delivery: role of erythrocyte count and oxygenation state of haemoglobin. J Physiol 572(1):295–305
23. Wolff CB (2007) Normal cardiac output, oxygen delivery and oxygen extraction. Adv Exp Med Biol 599:169–182
24. Ellsworth ML (2004) Red blood cell-derived ATP as a regulator of skeletal muscle perfusion. Med Sci Sports Exerc 36:35–41
25. Singel DJ, Stamler JS (2005) Chemical physiology of blood flow regulation by red blood cells: the role of nitric oxide and S-nitrosohaemoglobin. Annu Rev Physiol 67:99–145

26. Wan S, LeClerc JL, Vincent JL (1997) Cytokine responses to cardiopulmonary bypass: lessons learned from cardiac transplantation. Ann Thorac Surg 63:269–276

27. Kamler M, Goedeke J, Pizanis N, Milekhin V, Schade FU, Jakob H (2005) In vivo effects of hypothermia on the microcirculation during extracorporeal circulation. Eur J Cardiothorac Surg 28:259–265

28. De Backer D, Ortiz JA, Salgado D (2010) Coupling microcirculation to systemic hemodynamics. Curr Opin Crit Care 16:250–254

29. Gaehtgens P (1984) 'Regulation' of capillary haematocrit. Int J Microcirc Clin Exp 3(2):147–160